TSE Decontamination: Studies Relevant to Facility and Equipment Cleaning

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ZLB

for PPTA TSEWG

- ✓ NaOH and NaClO most commonly used in industry
- ✓ Inconsistent results in literature

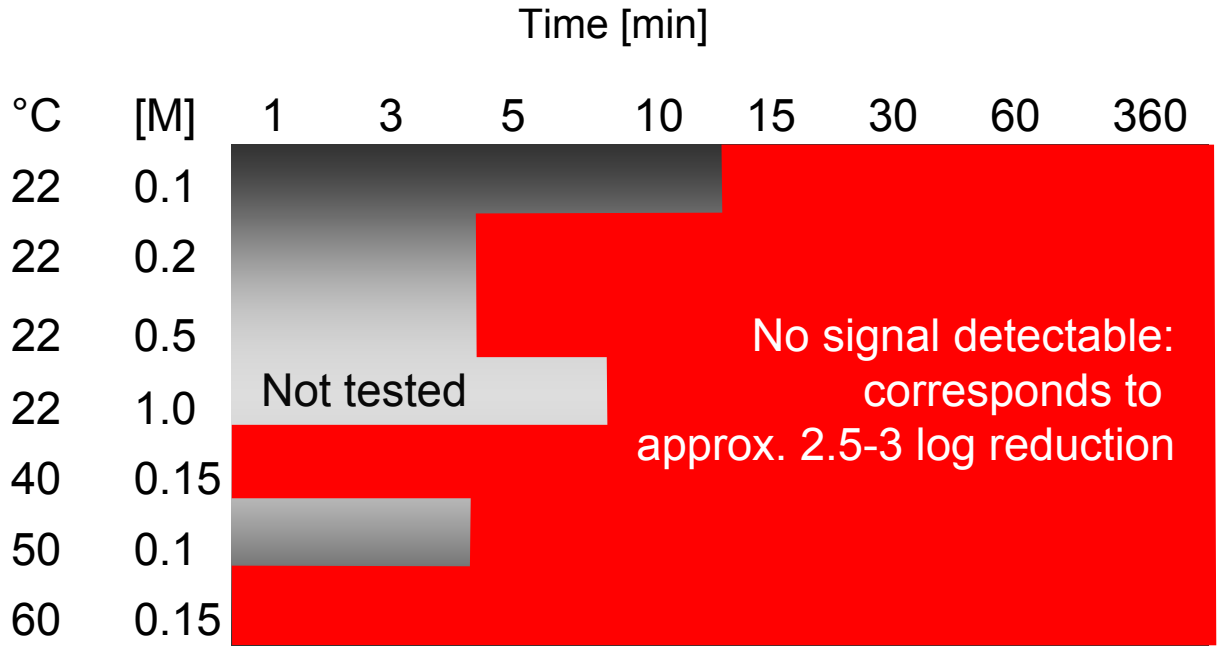
Compound	Time/ Temperature	Agent tested	Log ID_{50} change	Comment(s)
1 N sodium hydroxide	1 hr/RT	Hamster Scrapie	≥5.5	Effective
2 N sodium hydroxide	2 hr/RT	Hamster Scrapie	≥5.1	Effective
0.09 N sodium hydroxide	2 hr/RT/121 °C/1 hr	Hamster Scrapie	≥7.4	Effective
0.9 N sodium hydroxide	2 hr/RT/121 °C/1 hr	Hamster Scrapie	≥7.4	Effective
1 N sodium hydroxide	1 hr/RT	Hamster Scrapie	6.0	Effective
0.25 N sodium hydroxide	2 hr/RT	Mouse CJD	~1	Ineffective
1 N sodium hydroxide	2 hr/RT	Mouse CJD	2.9	Moderately effective

Reviewed by: Douglas Lee*, Wytold Lebing, Christopher Stenland, Stephen Petteway, Jr.

Downstream 27

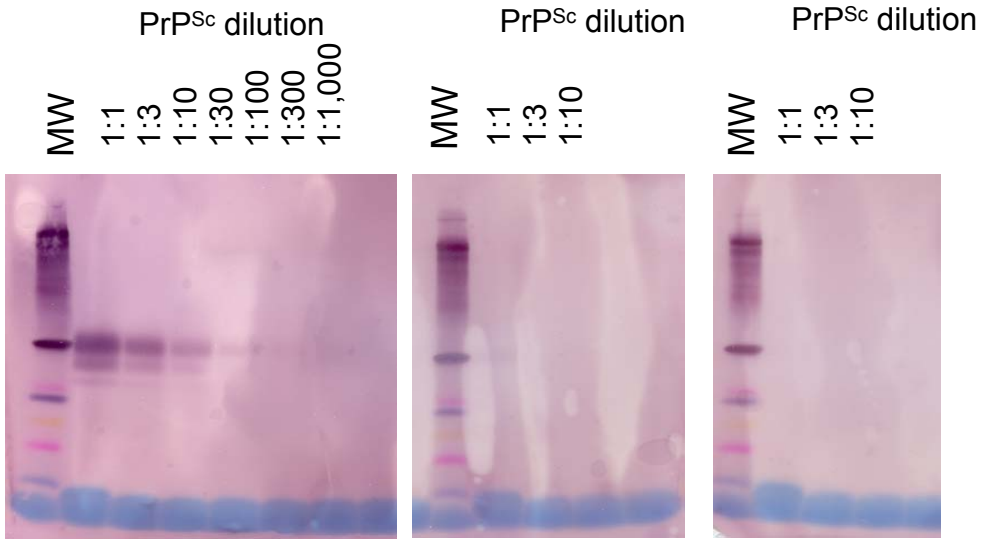
- ….The only methods that appear to be completely effective under worst case conditions are strong sodium hypochlorite solutions or hot solutions of sodium hydroxide … Taylor, Vet J. 159, 10 -17, 2000
- …Residual infectivity has been detected following treatment of 263K with 1M sodium hydroxide…(Diringer & Braig, Lancet 1, 439-40, 1989; Ernst & Race, J Virol Meth 41,193-202, 1993.
- etc…

Denaturation/Inactivation of Prions by NaOH-Treatment



263K hamster brain homogenate was incubated for various times at different temperatures and NaOH concentrations. PrP^{res} was determined by WB using the 3F4 antibody

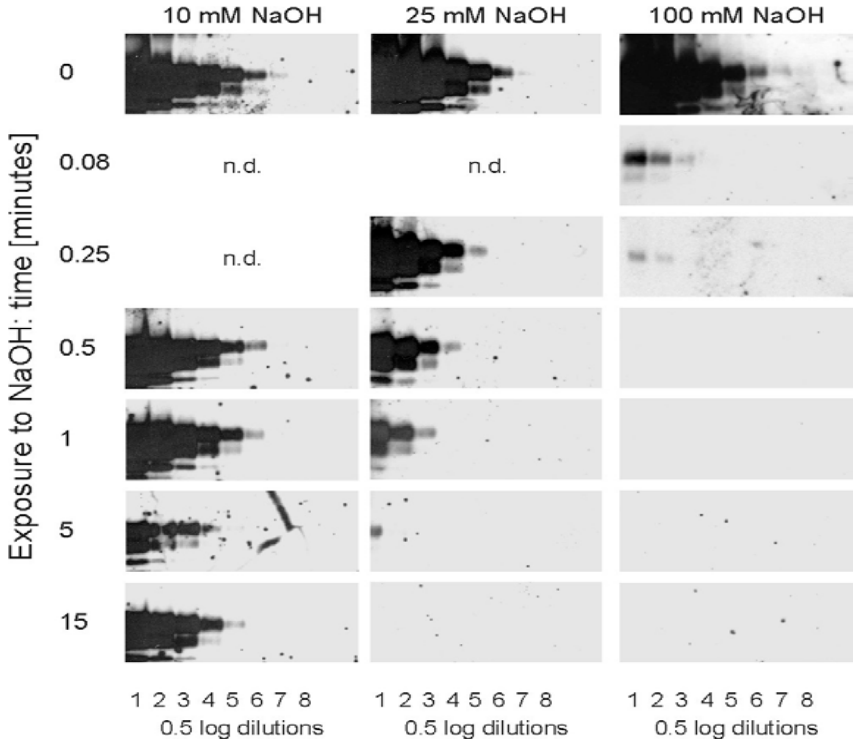
Denaturation/Inactivation of Prions by NaOH-Treatment



Microsomes purified from scrapie-infected hamster brain were incubated in 0.1 N NaOH at 22°C for 15 or 60 min

Denaturation/Inactivation of Prions by NaOH-Treatment

Kinetics of PrP^{res} disappearance after exposure of scrapie-positive hamster brain homogenate to NaOH at RT



Käsermann F, Kempf C. *J gen Virol in press*

Denaturation/Inactivation of Prions by NaOH-Treatment

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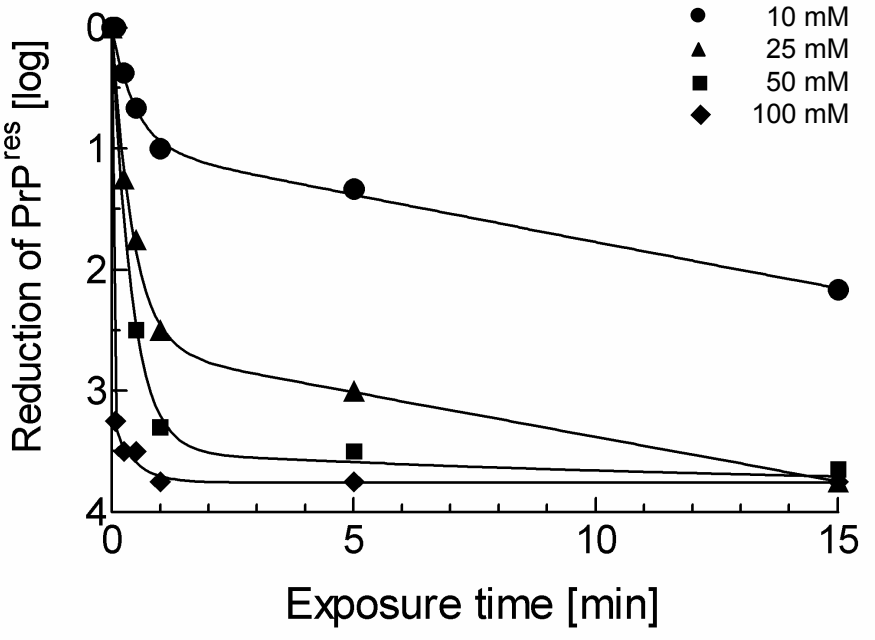


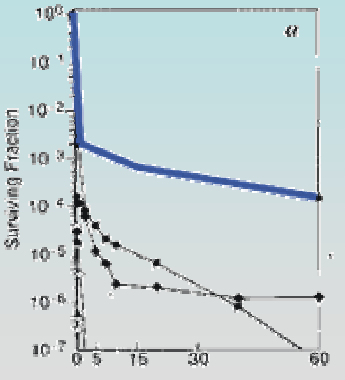
Käsermann F, Kempf C. *J gen Virol in press*

Denaturation/Inactivation of Prions by NaClO-Treatment

- PrP^{c} and PrP^{res} are both not detectable by the standard WB after short NaClO (100ppm) treatment even if no Proteinase K is used.
- The same was observed with recombinant PrP^{c}.
- Antibodies other than the 3F4 also do not result in a signal.
- Model proteins are not detectable after NaClO treatment either by Coomassie, silver or auro dye staining.

- Protein fragmentation is known to occur upon hypochlorite treatment by chloramine formation and nitrogen-centered radicals (Hawkins & Davies Biochem J 332, 617-625, 1998)

Inactivation of 263K by NaClO



Data:
Rohwer R.

Denaturation/Inactivation of Prions by NaOH/NaClO-Treatment

Treatment	Inactivation temperature or final concentration of chemical (wt/vol)	Decrease in log_{10} LD_{50} after exposure for			
		15 min	60 min	15 min	60 min
		CJD		Scrapie	
NaOH	0.1 *N*	4.8	4.8	5.0	6.0
	1.0 *N*	4.5	≥5.0†	6.0	≥6.8†
NaOH	0.25 *N*	4.7	4.8		
NaOH + 1% SDS	0.25 *N*	≥4.0	4.0		
NaOCl	0.5%			4.5	4.5
	1.0%			6.0	6.0
	2.5%		3.3	≥6.0	≥7.0

PAUL BROWN, ROBERT G. ROHWER, D. CARLETON GAJDUSEK

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Aventis

Aventis Behring

Evaluation of Sanitization

Sanitization of stainless steel surfaces was evaluated

- Hamster brain homogenate dried on stainless steel coupons
- Shaken in 0.1 N NaOH for 30 min, then purified water
- Recovery of PrP^{Sc} by swabbing
- Experiment performed in triplicate

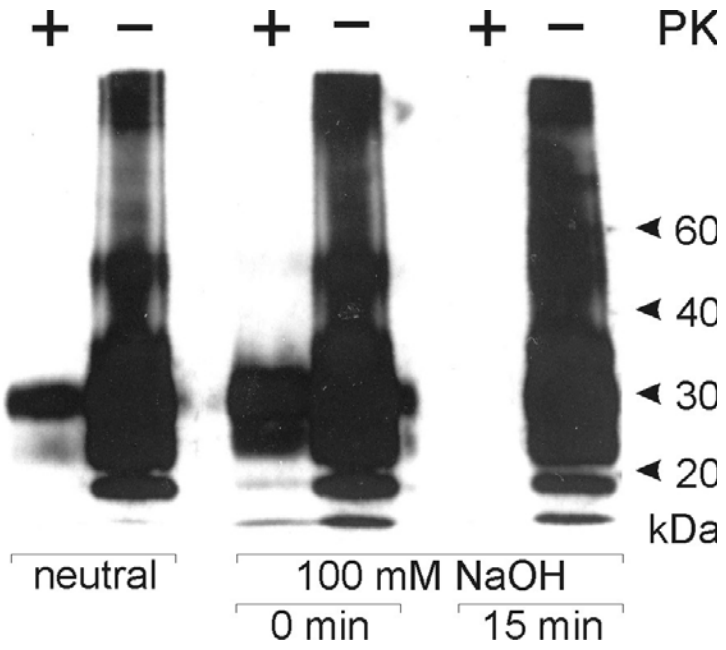
Results:

- Quantitative recovery of PrPSc from untreated coupon
- No residual PrPSc recovered from 0.1 N NaOH treated coupons

	Input Load [log_{10}]	Output Load [log_{10}]	Reduction [log_{10}]
Experiment 1	5.6	< 1.6	> 4.0
Experiment 2	5.1	< 1.6	> 3.5
Experiment 3	6.1	< 1.6	> 4.5

Evaluation of Sanitization

Iron powder previously loaded with scrapie-positive hamster brain homogenate was exposed to NaOH. Where indicated, iron powder samples were subsequently treated with PK (+). Prion proteins were dissolved in loading buffer and detected by Western blot analysis (equivalent of approx. 50 cm^2 / lane)



Käsermann F, Kempf C. *J gen Virol in press*

Infectivity of surface-bound mouse prions after various treatments

Inoculation	Sick/Total
1. Uninfected wires	
Untreated	0/3
2. Infectious wires	
Untreated	6/6
Sodium hydroxide (1M, 1 h, 25°C)	0/6
Formaldehyde (10%, 1 h, 25°C)	6/6
Guanidinium thiocyanate (4M, 16 h, 25°C)	0/6

E. Flechsig et al. Molecular Medicine 7(10): 679–684, 2001

Aventis

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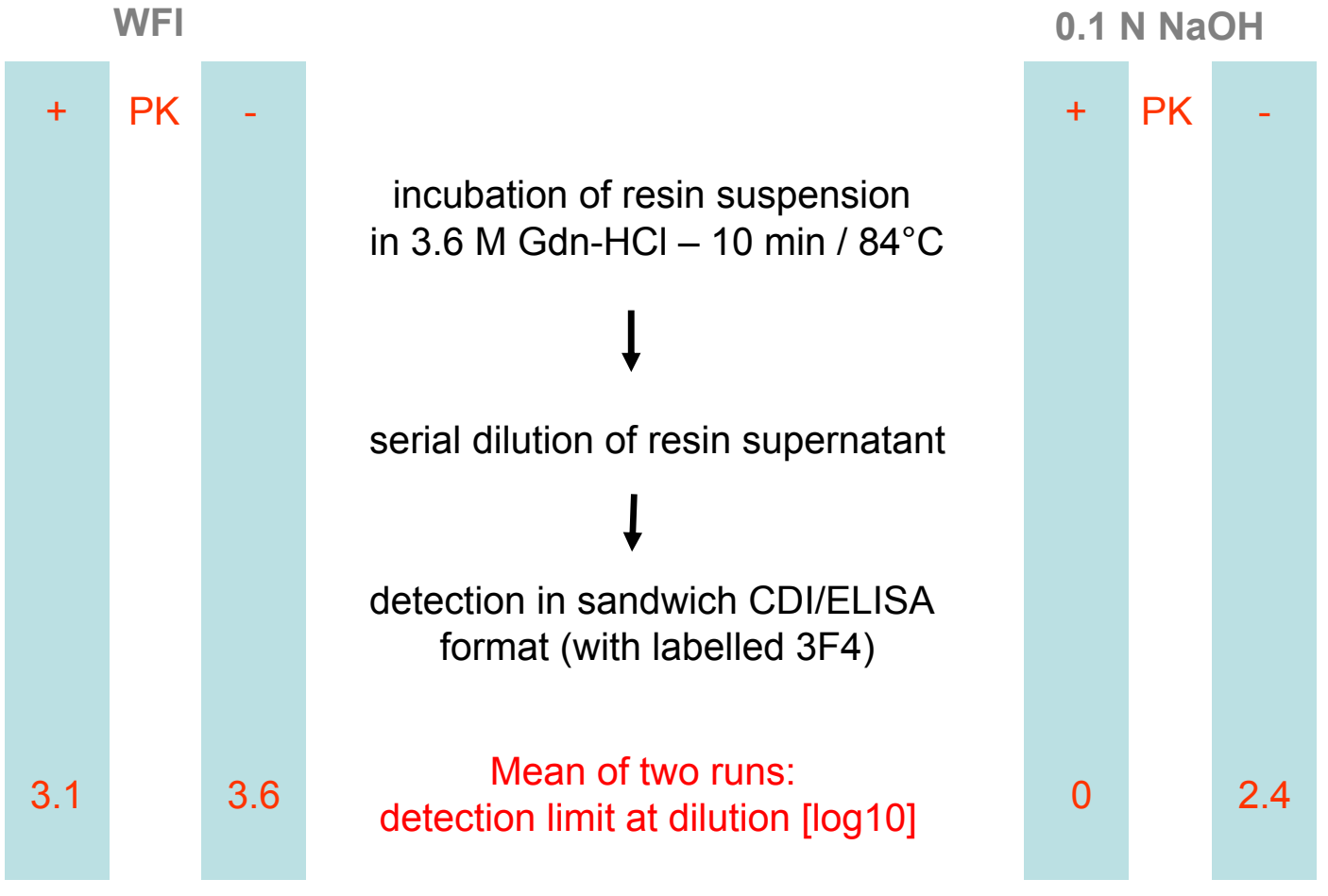
Evaluation of sanitization of DEAE-sepharose loaded with 263K microsomes by 0.1N NaOH or WFI (mock) for 5.5 hrs



Conclusions

- ✓ NaOH and NaClO treatment both destroy PrP^{res}
- ✓ Kinetics showed a rapid destruction of PrP^{res} even at low NaOH and NaClO concentrations
- ✓ Reduction of PrP^{res} in the range of up to >4.5 Log could be demonstrated
- ✓ These results are in agreement with many single point measurements based on infectivity data